Effects of yeast (*Saccharomyces cerevisiae*) and clinoptilolite administration on milk yield and some metabolic parameters in early lactation dairy cows*

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Abstract: This study was conducted to determine the effects of yeast (Saccharomyces cerevisiae) and clinoptilolite mixture on milk yield and some blood parameters related to metabolism in early lactation dairy cows. Twenty animals were selected from early lactation cows at 28 days in milk which were having similar milk yield and didn't show any symptom of metabolic diseases through transition period up to 28 days in milk. These cows were randomly assigned to two groups: control and treatment. Blend (yeast 60%, clinoptilolite 40%) was orally administrated 50 g/day to all treatment cows shortly after the afternoon milking through 4 weeks. The same basal ration was provided for all cows. Blood samples were collected from all cows on 21, 28, 35, 42 and 49th days of lactation and analyzed for NEFA, BHBA, total cholesterol, total protein, BUN, glucose, AST, ALT, GGT. Yeast and clinoptilolite administration increased milk yield ($p<0.01$) and this increase accelerated by proceeded weeks. Moreover, NEFA ($p<0.01$) and ALT ($p<0.01$) was increased but other blood parameters did not change in this study ($p>0.05$). Oral administration of yeast and clinoptilolite to early lactating dairy cows increased the milk yield but it had no significant effect on serum metabolites.

Keywords: Clinoptilolite, dairy cow, metabolic profile, milk yield, yeast

Erken laktosyon döneminde süt ineği rasyonlara ilave edilen maya (*Saccharomyces cerevisiae*) ve klinoptilolitin süt verimi ve bazı kan parametreleri üzerine etkisi

Öz: Bu çalışma, erken laktasyondaki süt ineklerinde maya (Saccharomyces cerevisiae) ve klinoptilolit karışımının süt verimi ile metabolizma ile ilişkili bazı kan parametreleri üzerine etkilerini incelemek amacıyla yapılmıştır. Geçiş periyodundan laktasyonun yirmi sekizinci gününe kadar benzer süt verimine sahip olmuş ve herhangi bir metabolic hastalık semptomu göstermemiş 20 baş erken laktasyon süt ineği laktasyonlarının 28.

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gününde seçilmiştir. Bu hayvanlar, kontrol ve uygulama olmak üzere rastgele iki gruba ayrılmıştır.. Karışım (%40 klinoptilolit; %60 maya), uygulama grubundaki tüm ineklere 50 g/gün dozunda öğle sağımını müteakip 4 hafta süresince içirilmiştir. Tüm ineklere benzer bazal rasyon verilmiştir. Laktasyonun 21, 28, 35, 42 ve 49 günlerinde tüm ineklerden kan örnekleri alınmış ve NEFA, BHBA, total kolesterol, total protein, BUN, glikoz, AST, ALT, GGT analizleri gerçekleştirilmiştir. Maya ve klinoptilolit uygulaması süt verimini artırmış (P<0,01) ve bu artış haftalar içerisinde ivmesine devam etmiştir. Bunun yanında NEFA (P<0.01) ve ALT (P<0.01) düzeyleri de artmış ancak diğer kan parametreleri gruplar arası değişim göstermemiştir (P>0,05). Maya ve klinoptilolitin erken laktasyon süt ineklerine içirilmesi, süt verimini artırırken; serum metabolitleri üzerine herhangi bir etki göstermemiştir.

Anahtar sözcükler: Klinoptilolit, maya, metabolik profil, süt ineği, süt verimi

Introduction

Yeast products are commonly originated from *Saccharomyces cerevisiae* and widely being used in commercial dairy farms to improve milk yield. *Saccharomyces cerevisiae*, an active dry yeast, which is a common type of yeast and is most widely used as a feed additive in ruminants. These microorganisms have limited ability to reproduce within the rumen (14). Some researchers explained the beneficial effects of yeast cultures that altered the rumen environment, through increasing the number and activity of rumen cellulolytic bacteria and minimising the dramatic changes in ruminal pH (10, 31). It is thought that yeast products affect the rumen microbial population, by changing volatile fatty acids (VFA) production in the rumen which ultimately results in increased milk production along with an increase in milk fat and milk protein yields from lactating dairy cows (7, 25). Researchers are continuing in this area to determine the specific mechanism of yeast cultures on microbial fermentation in rumen environment (26).

Yeast supplementation appears to be beneficial shortly before parturition and during high yielding phases of lactation (6, 18). Because these periods are characterized negative energy balance due to decreased Dry Matter Intake (DMI) especially last weeks of gestation. Most of the studies which used yeast supplementation to early lactation dairy cows, improved DMI (30), milk yield (13, 22, 30, 32), and milk composition (30, 32). Although some studies suggested no response of yeast supplementation to a diet of early lactating dairy cows (3, 8). Some researchers suggested that several factors, including the stage of lactation, type of forage feed, feeding strategy, and forage to concentrate ratio, are likely to affect the response of yeast cultures in dairy cows (22).

Clinoptilolite is a natural clay mineral that belongs to the zeolite group. Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations that have infinite three-dimensional structures (24). Clinoptilolite is a hydrated aluminosilicate - $(Na_4K_4)(Al_4Si_4O_{96})$ $24H_2O$ (4) and characterized by the ability to lose and gain water reversibly, to absorb molecules of appropriate diameter or acting as molecular sieves, and to exchange their constituent cations without major change of their structure (ion-exchange property) (16). Due to their physical and chemical properties zeolites, especially clinoptilolite, are used in animal nutrition mainly to absorb aflatoxins, improve performance and health status (15, 21). In some cases, feeding clinoptilolite improved the hematological and biochemical parameters and histological picture of both liver and kidneys in rats (1). In addition, a clinoptilolite-rich zeolite in sheep diet could show some protective effects on rumen microbiota against the organophosphate poison (15, 19, 27). On the other hand; some researchers claimed, clinoptilolite feeding can make some positive effects on milk yield in dairy cows (28).

The current study was conducted to determine the effects of yeast (*Saccharomyces cerevisiae*) plus clinoptilolite on milk yield and some blood parameters related to metabolism in early lactation dairy cows.

Materials and Methods

Animals and experimental design: This study was conducted in Nigtas Dairy Company, Niğde-Turkey having. 1600 dairy cows, heifers, and calves. At the beginning of the study, all early lactating dairy cows were monitored for the milk yield and metabolic diseases for the first 28 days after parturition. Twenty, healthy Holstein cows (didn't show any symptom of the metabolic diseases through transition period) were selected and assigned to two groups on the twenty-eighth day of lactation. Mean milk yield in all the cows was similar (36 L/1 L). Yeast and clinoptilolite mixture (Y+C) (50 g/day/cow, yeast 60%, clinoptilolite 40%, Rumencure®, Ideal Feed Additives, Afyonkarahisar/Turkey) was orally administrated to all treatment cows shortly after the afternoon milking for 30 days. Control cows received water administration orally to perform same stress conditions. All cows were kept in the same paddock and consumed the same diet. The diet was designed to meet NRC (17) requirements. Diet was split up into three equal parts in a day and fed as TMR (Total Mix Ration). Every feed in TMR was analysed for crude fibre, crude protein, ether extract, crude ash and dry matter according to Weende Analysis System, besides ADF and NDF according to Goering and Van Soest (9). Cows were milked three times daily at 04:30 am, 12:30 pm and 08:30 pm daily milk yield was measured and recorded for all cows.

Table 1: Feed ingredients and chemical composition of ration

Tablo 1: *Yem içerikleri ve rasyonun kimyasal kompozisyonu*

Ingredients	(DM%)
Concentrate mixture	27.8
Corn Silage	25.45
Alfalfa	14.8
Barley	8.4
Citrus Pulp	8
Soybean Meal (48% CP)	5.7
Cottonseed	5.5
Corn Gluten Meal	4.2
Bicarbonate	0.15
Chemical composition of ration	(DM%)
DM	57
Crude Protein	17.2
Microbial Protein	11.6
Net Energy Lactation (Mcal/kg)	1.61
NDF	39.35
ADF	21.42
Calcium	0.78
Phosphorus	0.38

Blood samples and analysis: Blood samples were collected in empty vacutainer tubes via vena jugularis from all cows on 28, 35, 42 and 49th days of lactation, centrifuged at 5000 × g for 10 min. Then, serum was collected, stored at -15°C and analysed for NEFA, BHBA, TChol, TP, BUN, glucose, AST, ALT, GGT by using commercial kits in ELISA reader (Chemwell® Model 2910, Awareness Technology Inc., Palm City, FL, USA).

Statistical analysis: PASW Statistics (version 18.0, SPSS, Chicago, IL) was used for data analyses. The Mann-Whitney U-test was used to compare mean differences between groups. Wilcoxon's signed ranks test was performed after the Friedman test to determine where significance occurred for within group variables. A significance level of P<0.05 was used. To avoid type 1 Alfa error; Bonferroni correction was used for Wilcoxon's signed rank test.

Results and Discussion

AST, GGT, TChol, TP, BUN, BHBA and glucose levels were similar between groups, throughout the study (P>0.05 Table 2). ALT level for the Y+C was higher than control in only last week of the experiment (P<0.05, Table 2). The NEFA level of the Y+C was lower at the second week and higher at last week of study than control (P<0.01, Table 2). But except these differences, all NEFA levels were acceptable and none of them increased to ketotic levels. None of the experimental cows showed any signs of metabolic disorders. Similar to these results, some researchers (11) found no effect of supplemental yeast on serum metabolites in dairy cows.

Table 2: Serum biochemical parameters

Tablo 2: *Serum biyokimyasal parametreleri*

AST	Y+C	71.73±3.15	72.54±2.45	75.17±1.25	75.70±1.87	78.93±2.05	P>0.05
(U/L)	Control	70.86±3.59	71.86±1.67	76.71±1.54	76.65±2.54	78.20±2.01	P>0.05
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	
ALT	Y+C	30.29±1.18	31.25±1.14	32.14±1.61	31.81±0.95	34.27±1.41[A]	P>0.05
(U/L)	Control	31.52±1.24	31.52±1.32	32.52±1.23	32.16±1.37	29.92±1.40[B]	P>0.05
	P	P>0.05	P>0.05	P>0.05	P>0.05	P<0.05	
GLU	Y+C	49.33±2.03	47.32±2.32	51.96±1.99	49.36±2.06	49.18±1.34	P>0.05
(mg/dL)	Control	48.65±1.84	43.25±0.99	46.97±1.32	46.42±1.21	49.62±1.49	P>0.05
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	
CHO	Y+C	108.31±1.68	110.58±1.32	116.65±2.19	113.09±3.61	114.88±1.88	P>0.05
(mg/dL)	Control	112.34±1.70	111.32±1.54	111.82±2.77	111.01±1.44	115.12±1.93	P>0.05
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	
TP	Y+C	6.58±0.10	6.52±0.11	6.45±0.08	6.41±0.08	6.60±0.09	P>0.05
(g/dL)	Control	6.62±0.09	6.58±0.08	6.60±0.11	6.43±0.08	6.50±0.11	P>0.05
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	
GGT	Y+C	20.62±0.72	20.87±0.69	21.42±0.91	20.81±0.41	21.00±0.60	P>0.05
(U/L)	Control	20.97±0.44	20.65±0.48	21.52±0.58	21.54±0.42	20.40±0.40	P>0.05
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	
URE	Y+C	22.57±0.76	22.35±0.57	21.77±0.62	21.32±0.48	22.21±0.58	P>0.05
(mg/dL)	Control	22.47±0.66	22.17±0.61	21.97±0.77	21.25±0.45	21.30±0.45	P>0.05
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	
NEFA	Y+C	0.23±0.02	0.22±0.01	0.21±0.01[A]	0.20±0.02	0.22±0.01[A]	P>0.05
(mmol/L)	Control	0.23±0.01[a]	0.22±0.02[a]	0.28±0.01[B,bc]	0.20±0.01[bd]	0.18±0.01[B,bd]	P<0.001
	P	P>0.05	P>0.05	P<0.01	P>0.05	P<0.01	
BHBA	Y+C	0.63±0.01	0.63±0.01	0.61±0.01	0.65±0.02	0.66±0.02	P>0.05
(mmol/L)	Control	0.62±0.01	0.62±0.01	0.64±0.01	0.63±0.01	0.64±0.01	P>0.05
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	

The values represented by mean±SE; Differences represented by A, B between groups and a,b within the group.

Table 3: Milk yields

Tablo 3: *Süt verimleri*

	0	1	2	3	
Y+C	36.60±0.27[a]	38.60±0.24[A,b]	39.65±0.35[A,b]	41.55±0.59[A,b]	P<0.001
Control	36.75±0.63[a]	36.95±0.50[B,b]	37.45±0.35[B,b]	38.15±0.37[B,b]	P<0.01
	P>0.05	P<0.05	P<0.01	P<0.01	

The values represented by mean±SE; Differences represented by A, B between groups and a,b within the group.

Y+C administrated cows produced more milk than control cows in first two weeks (P<0.05), and in last two weeks (P<0.01). Also, increase the milk yield by week was higher in Y+C than control (Table 3). Similar to these findings, yeast feeding improved milk yield in dairy cows in some cases (20, 23, 31, 32). However, several studies indicated supplemental yeast had no beneficial effect on milk yield in dairy cows (3, 8, 26, 29).

Several factors affect the response of yeast supplementation; such as the type of forage given (11), the forage: concentrate ratio (22), stage of lactation (6, 7), and yeast strain and viability (2, 5, 18). Several researchers (28) indicated that dietary supplementation of clinoptilolite increased milk yield in dairy cows. But inconsistently, others (4) demonstrated that supplemental clinoptilolite did not affect milk yield. Periparturient cows fed clinoptilolite had fewer cases of clinical ketosis during the first month after calving and a higher total milk yield (12). Besides consistent to findings of this study, feeding the cows with clinoptilolite for a long period had no apparent adverse effects on their liver function, and did not significantly affect the concentrations of glucose, ketone bodies, BUN and total proteins in their serum.

Conclusion

From the results of this study, it is concluded that oral administration of yeast and clinoptilolite to early lactating dairy cows increased the milk yield but it had no significant effect on serum metabolites. Further researches are needed to clearly describe this synergetic effect between yeast and clinoptilolite.

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